International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-2, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

# Base Degradation Monitoring of Dolasetron Mesylate by UV Spectrophotometric Method

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Manuscript No: IJPRS/V4/I2/00126, Received On: 13/06/2015, Accepted On: 26/06/2015

## ABSTRACT

A simple and fast stability indicating spectrophotometric method has been developed and validated for estimation of Dolasetron mesylate that can be used for routine analysis. Dolasetron mesylate is a potent antinauseant and antiemetic agent. It is a serotonin 5-$HT_3$ receptor antagonist. Spectrophotometric detection of Dolasetron mesylate shows maximum absorbance at 285 nm. Alkali degradation monitoring of Dolasetron mesylate was performed by first derivative spectrophotometric method at 229 nm. The method was found to be linear in concentration range 10-100µg/ml. Mean assay of Dolasetron mesylate was found to be 101.48%. The method validated for parameters such as linearity, range, precision, robustness, sensitivity and accuracy according to ICH Q2R1 (International Conference on Harmonization). When stress degradation studies were carried under ICH recommended stress condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis according to ICH Q1AR2, it was observed that presence of Dolasetron degradation product could be detected by first derivative UV method.

## KEYWORDS

Dolasetron Mesylate, UV Spectroscopy, Stability Indicating Method

## INTRODUCTION

Dolasetron mesylate, chemically (2α,6α,8α,9aβ)-octahydro-3-oxo-2,6-methano-2*H*-quinolizin-8-yl-1*H*-indole-3-carboxylate mono methane sulfonate monohydrate, is an highly specific and selective serotonin subtype 3 (5-HT) receptor antagonist (Fig: 1), used to treat nausea and vomiting in chemotherapy[1,2]. Its main effect is to reduce the activity of the vagus nerve, which is a nerve that activates the vomiting center in the medulla oblongata. Dolasetron mesylate is official in USP30-NF25 with HPLC method for its estimation[3].

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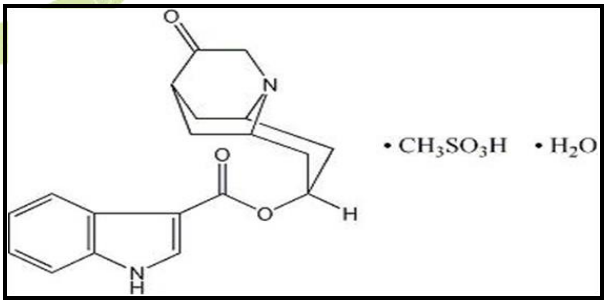


Figure 1: Chemical structure of Dolasetron mesylate

Literature survey reveals following methods reported viz., simple RP-HPLC method[4-7] for quantitative analysis of Dolasetron mesylate, LC-MS and GC-MS[8,9] methods for determination of Dolasetron and its reduced metabolite in human plasma. We have reported stability indicating

HPTLC method[10] for determination of Dolasetron mesylate.

But these chromatographic methods are complex, expensive, time consuming as compared to simple UV spectrophotometric method. Therefore, it was considered necessary to develop Stability-indicating UV spectro-photometric method for determination of Dolasetron mesylate. The present work involves stress degradation as per ICH Q1A (R2) and Q1B[11,12] for developing simple, fast, stability indicating UV spectrophotometric method. Distilled water was used in entire analysis, so developed method is cost effective. The method was validated as per the ICH guidelines Q2 R1.[13]

We had observed that the peak for product of alkaline degradation was well resolved in HPTLC studies. Hence we tried to monitor alkaline degradation of Dolasetron mesylate by derivative method in UV spectroscopy.

## MATERIAL AND METHODS

### Chemicals and Reagents

Dolasetron mesylate was provided as a gift sample by Emcure Pharmaceuticals, Pune. Drug was used as such, without any further purification. Double-distilled water used as solvent.

### Instruments

Double beam UV-VIS Spectrophotometer (Jasco V-550) was used for study. Photostabilty chamber (Nutronics-NEC103RSPI) was used for photostability studies. All weighing was done on Shimadzu balance (Model AY-120).

### Preparation of Standard Stock Solution of Dolasetron Mesylate

Standard stock solution of Dolasetron mesylate was prepared by dissolving accurately weighed quantity of Dolasetron mesylate 10 mg using water in 10 mL volumetric flask. This solution was suitably diluted to get 100µg/ml solution.

### Determination of Absorption Maxima

The spectrum was recorded for standard solution (10 µg/ml) scanned against solvent (Water) as blank between 200-400 nm. The 285 nm absorption maxima were selected for analysis (Fig: 2).

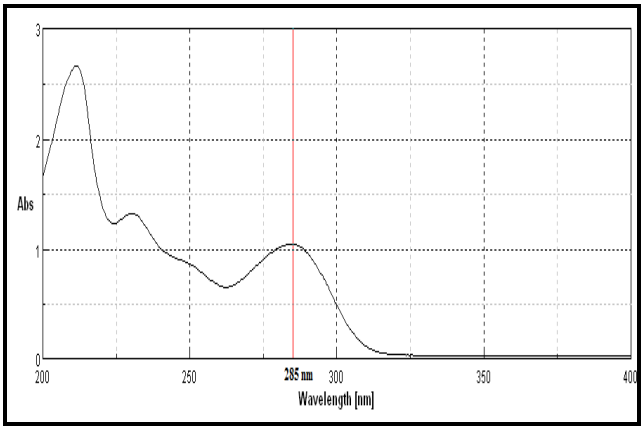


Figure 2: UV Spectrum of Dolasetron mesylate (60 µg/ml)

### Calibration Curve for Dolasetron mesylate

Various aliquots of standard stock were taken and diluted to 10 ml with water to obtain solution having concentration range 10, 20, 40, 60, 80 and 100µg/ml. Then absorbance of all these solution measured at $\lambda_{max}$ 285 nm and the corresponding value were plotted against concentration to obtain calibration curve (Fig: 3).

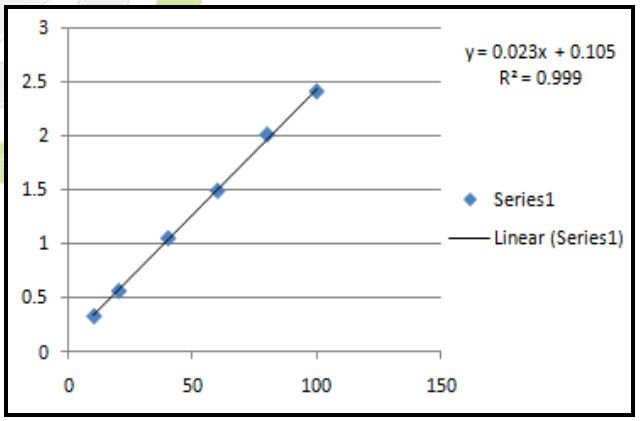


Figure 3: Calibration curve for Dolasetron mesylate (10-100 µg/ml)

## RESULTS AND DISCUSSION

### Forced Degradation Studies

Forced degradation studies were carried under ICH recommended stress condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared: The blank subjected to stress in the same manner as the drug solution, working

standard solution of Dolasetron mesylate subjected to stress condition. Dry heat and photolytic degradation were carried out in solid state. The optimized stress conditions reported by us[10] were used. The results of all stress conditions are given in Table 1.

Table 1: Summary of stress degradation study

| Stress Conditions | % Recovered | % Degradation |
|---|---|---|
| Acid (0.1N HCl for Overnight) | 82.75 | 17.25 |
| Alkali (1N NaOH for 2 hours) | 88.03 | 11.97 |
| Neutral (At 80°C for 48 hours) | 80.93 | 19.07 |
| Oxidative (10% $H_2O_2$ for 1 hours) | 88.78 | 11.22 |
| Thermal(80°C for 24 hours) | 74.05 | 25.95 |
| Photo Stability (UV, 200 Watt Hrs/Square Meter) (Fluorescence 1.2 Million Lux. Hrs) | 81.47<br>66.21 | 18.53<br>33.39 |

## Acid Hydrolysis

1 ml working standard solution of Dolasetron mesylate (600µg/ml) was mixed with 1 ml of 0.1 N HCL and volume make up to 10 ml with water (i.e 60µg/ml). Solution was kept for overnight and absorbance was measured at 285 nm.

## Alkali Hydrolysis

1 ml working standard solution of Dolasetron mesylate (600µg/ml) was mixed with 1 ml of 1N NaOH and volume make up to 10 ml with water (i.e 60µg/ml). Solution was kept for 2 hours and absorbance was measured at 285 nm (Fig: 4).

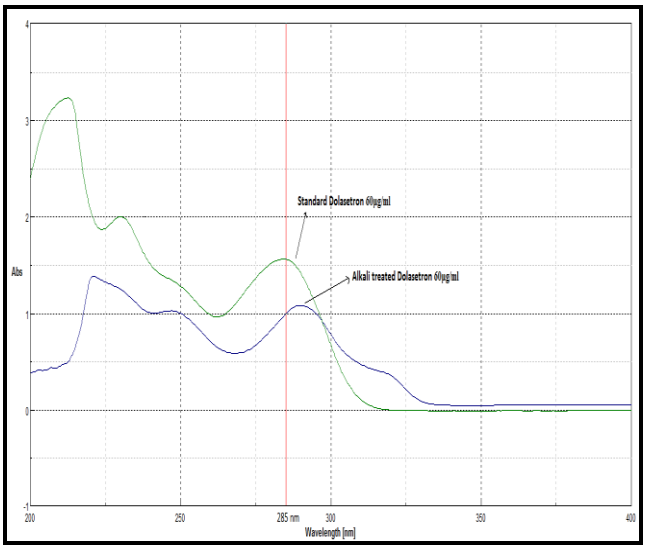


Figure 4: Overlaid spectra of standard Dolasetron and Alkali treated Dolasetron mesylate at 285 nm

## Alkali Degradation Monitoring by First Derivative Method at 229 nm

Alkali degradation monitoring of Dolasetron mesylate was performed by using first derivative method at 229 nm. The 1st derivative spectrum of standard Dolasetron mesylate crosses zero (i.e X-axis) at this $\lambda_{max}$ of 229 nm whereas, absorbance of alkali treated Dolasetron mesylate increases linearly with concentration at this $\lambda_{max}$ (Figure: 5).

Absorbance of degradation product was found to linearly increase in concentration range 40-80 µg/ml (Table 2).

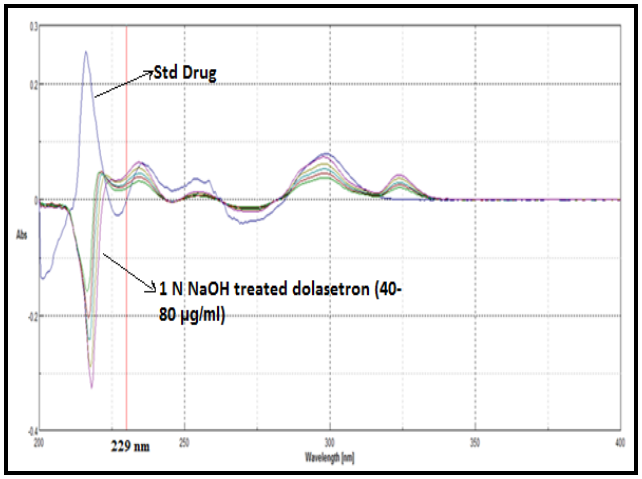


Figure 5: Base degradation monitoring of Dolasetron mesylate by derivative method at 229nm

Table 2: Absorbance of stress degraded sample by 1st Derivative method

| Sr. No | Alkali treated Dolasetron (1N NaOH for 2 Hr) | Absorbance at 229 nm |
|---|---|---|
| 1. | 40 µg/ml | 0.01805 |
| 2. | 50 µg/ml | 0.02399 |
| 3. | 60 µg/ml | 0.02791 |
| 4. | 70 µg/ml | 0.03439 |
| 5. | 80 µg/ml | 0.03945 |
| | | Y= 0.000x - 0.003<br>$R^2$= 0.996 |

**Neutral Hydrolysis**

1 ml working standard solution of Dolasetron mesylate (600µg/ml) was mixed with 9 ml water (i.e 60µg/ml). Solution was kept for 48 hours and absorbance was measured at 285 nm.

**Oxidative Hydrolysis**

1 ml working standard solution of Dolasetron mesylate (600µg/ml) was exposed with 1 ml of 10% $H_2O_2$ and volume was made up to 10 ml with water (i.e 60µg/ml). Solution was kept for 1 hour and absorbance was measured at 285 nm (Figure: 6).

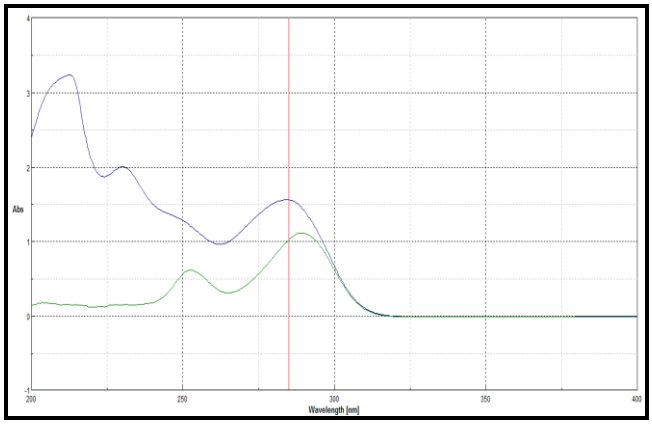

Figure 6: Overlaid spectra of standard Dolasetron and Peroxide treated Dolasetron mesylate at 285 nm

**Degradation under Dry Heat**

Dry heat studies were performed by keeping drug sample in oven at 80°C for 24 hrs. 10mg was weighed & dissolved in 10ml water and was suitably diluted to get concentration of 60µg/ml and absorbance was measured at 285 nm.

**Photo Degradation Study**

The photo stability study of the drug was determined by exposing the drug under UV light, which provides illumination of NLT 200 watt hr/$m^2$ followed by cool white fluorescence light of NLT 1.2million Lux-Hr. After each exposure accurately 60µg/ml photo exposed solution made for absorbance at 285nm.

**Analytical Method Validation**

UV spectrophotometric method for determination of Dolasetron mesylate was validated for the linearity, precision, assay, accuracy, LOD and LOQ at $\lambda_{max}$ 285 nm according to ICH Q2 (R1) guidelines.

**Linearity and Range**

Calibration curve was found to be linear in the range from 10 to 100 µg/ml (Fig: 7). It was constructed with 6 different concentrations (10, 20, 40, 60, 80 and 100). Each concentration was analysed in 6 replicates. The regression equation was y = 0.023x + 0.105, ($R^2$ =0.999).

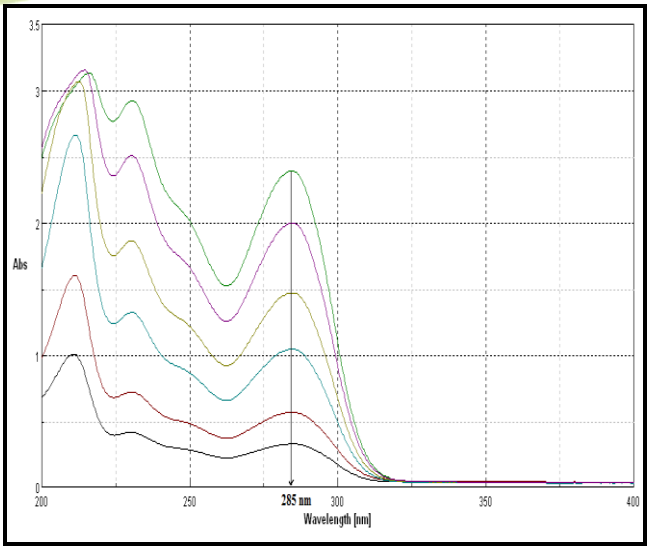


Figure 7: Linearity of Dolasetron mesylate (10-100µg/ml)

**Precision**

**Intra-day and Inter-day**

The intra-day and inter-day precision study of Dolasetron mesylate was carried out by estimating different concentrations of Dolasetron mesylate (20, 40, 60 µg/ml), three times on the same day and on three different days and the results were reported in terms of % RSD (Table 3).

**Accuracy**

Accuracy was assessed by determination of the recovery of the method by standard addition to drug-excipients blend at 3 different concentration levels 80%, 100%, and 120%. Initial concentration of sample chosen was 40µg/ml. The drug concentrations of Dolasetron mesylate were calculated by using linearity equation. Each concentration was analyzed 3 times and average recoveries were measured. The results are reported in Table 4.

**Limit of Detection and Limit of Quantitation (LOD and LOQ)**

The LOD (limit of detection) and LOQ (limit of quantitation) of Dolasetron mesylate were estimated from the standard deviation of the response and the slope of the calibration curve. LOD and LOQ were calculated according to the formula 3.3 σ/S and 10 σ/S respectively.

Where;

σ is the standard deviation of lowest response.

S is the slope of the calibration curve.

Applying this formula, LOD and LOQ were found to be 0.53 µg/ml and 1.60 µg/ml respectively and results are clearly indicated in Table 5.

**Robustness**

Robustness of the method was determined by carrying out the analysis of 20, 40, 60 µg/ml

Table 3: Intra-day and Inter-day Precision of Dolasetron mesylate

| Concentration (µg/ml) | Intra-day Precision | | Inter-day Precision | |
|---|---|---|---|---|
| | SD of Absorbance | %RSD | SD of Absorbance | %RSD |
| 20 | 0.01 | 1.93% | 0.005 | 0.8% |
| 40 | 0.002 | 0.2% | 0.022 | 2.0% |
| 60 | 0.0008 | 0.05% | 0.009 | 0.6% |

Table 4: Recovery studies of Dolasetron blend formulation

| Sr. No | Conc. Level | Dolasetron Blend (µg/ml) | Amount of Std. Dolasetron added in µg/ml | % Recovery | % RSD |
|---|---|---|---|---|---|
| 1. | 80% | 40 µg/ml | 32 µg/ml | 100.6 | 1.25 |
| 2. | 100% | 40 µg/ml | 40 µg/ml | 103.3 | 1.63 |
| 3. | 120% | 40 µg/ml | 48 µg/ml | 102.1 | 1.18 |

concentration of Dolasetron mesylate under conditions during which slight change in Wavelength was done and % RSD was determined, (Table 6).

Table 5: LOD and LOQ of Dolasetron mesylate

| Parameters | Dolasetron mesylate |
|---|---|
| SD | 0.0037 |
| Slope | 0.023 |
| LOD (µg/ml) | 0.53 |
| LOQ (µg/ml) | 1.60 |

Table 6: Robustness of Dolasetron mesylate

| Sr. No | Parameters | Robust condition | % RSD |
|---|---|---|---|
| 1 | Wavelength (285 nm) ± 1 nm | A) 284 nm | 0.05% |
| | | B) 286 nm | 0.02% |

Table 7: Summary of validation parameters

| Sr. No | Parameters | Results of Dolasetron mesylaye |
|---|---|---|
| 1 | Linearity Range | 10-100µg/ml |
| 2 | Regression equation & Correlation coefficient | Y= 0.023x + 0.105<br>$R^2$= 0.999 |
| 3 | Precision<br>Intra-day<br>Inter-day | <br>%RSD = 0.72<br>%RSD = 1.13 |
| 4 | Assay | 101.48% |
| 5 | Accuracy | 102.0% |
| 6 | LOD (µg/ml) | 0.53 |
| 7 | LOQ (µg/ml) | 1.60 |
| 8 | Robustness | Robust |

## DISCUSSION

A stability indicating assay method was performed at 285 nm. Presence of alkali degradation product could be monitored at 229 nm (In 1st derivative method). The stability study indicates that Dolasetron degrades upon treating the drug with acid, alkali, neutral hydrolysis, Oxidative, thermal and photolytic conditions. The extent of degradation determined by this method matches fairly well with results of HPTLC[10] method reported by us.

No stability indicating UV spectrophotometric method has been reported for Dolasetron mesylate. The present work involves development of simple, fast, cost effective stability indicating UV method for routine analysis of Dolasetron mesylate.

## CONCLUSION

Proposed spectrophotometric method is simple and fast for the estimation of Dolasetron mesylate. Only HPLC and HPLC-MS methods have been reported which are costlier and time consuming than spectrophotometric method for analysis. The first derivative method was easily applicable for degradation monitoring of Dolasetron mesylate in alkali degradation conditions. In the proposed method simple distilled water used as solvent, which allows the analysis of a large number of samples at low cost. Thus the proposed method is simple, economic and has been validated as per the ICH guidelines. Therefore the proposed method can be successfully used in laboratories to estimate Dolasetron mesylate.

## ACKNOWLEDGEMENT

The authors are thankful to Emcure Pharmaceutical Pvt. Ltd Kurkumbh, Pune for providing gift sample of Dolasetron mesylate and also to the Management & Principal of AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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